The Identity of *Flindersia pimenteliana* and *F. oppositifolia* (Rutaceae): Evidence from DNA sequences

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Summary

Scott, Kirsten D., Harris, Wayne K. & Playford, Julia. (2000). The Identity of *Flindersia pimenteliana* and *F. oppositifolia* (Rutaceae): Evidence from DNA sequences. *Austrobaileya* 5(4): 667–669. Nucleotide sequencing of two independent genomic regions has shown that two *Flindersia* species, *F. oppositifolia* F.Muell and *F. pimenteliana* F.Muell were genetically indistinguishable. This coupled with supporting morphological observations by Hartley (1969) and Whiffin (1982) has lead us to reassess the taxonomic status of the two species. The new recombination is *Flindersia pimenteliana* F.Muell. forma *oppositifolia* (F.Muell.) K. D. Scott, W. K. Harris & J.Playford, comb. & stat. nov.

Key words: Rutaceae, *Flindersia pimenteliana, Flindersia oppositifolia* DNA sequences, systematics, Australia

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Introduction

The genus *Flindersia* R.Br. is a predominantly Australian group with one species in the Moluccas, one in New Caledonia and four in New Guinea (Hartley 1969). The genus has 17 species in total, and they are found from rainforest through to semi-arid habitats. During a study on the molecular phylogeny of the genus (Scott *et al.*, in press) it became apparent that *F. oppositifolia* and *F. pimenteliana* were anomalous, in that their nucleotide sequences in two different genomic regions were identical. This coupled with the observations and conclusions of Hartley (1969) that the two species were very closely related and Whiffin's (1982) conclusion that *F. oppositifolia* (F.Muell.) was a 'highly derived montane form' of *F. pimenteliana* F.Muell. has lead us to reassess the taxonomic status of the two species.

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Methods

DNA was extracted from 3 individuals of *F. oppositifolia* and *F. pimenteliana* from both fresh and herbarium material, using the extraction protocol of Scott and Playford (1996). PCR of both the chloroplast and the nuclear DNA fragments was in a 25µl volume containing: 1.5 mM $MgCl_2$, 10 mM KCl, 20 mM Tris-HCl (pH8.7), 10 mM $(NH_4)_2$ SO_4, 5 µl of Qmix (Qiagen, Clifton Hill), 0.2 mM each dNTP, 0.25 µM each primer (chloroplast primers e and f; Taberlet *et al.* 1991: nuclear ITS-1 primers; GN1 - Scott and Playford 1996, and C1–5' TAC GTT CTT CAT CGA TGC GA 3' G.Graham personal communication), 1.25 U *Taq* polymerase (Qiagen, Clifton Hill) and 20 ng DNA. Thermal cycling was in a FTS-1 Thermal Sequencer (Corbett Research, Mortlake) on a program of 94°C for 20 s, 55°C for 20 s, and 72°C for 90 s, for 35 cycles. 3 µl of each reaction were run on a 1% TBE agarose gel to confirm amplification. The remaining PCR product was purified by the addition of an equal volume of

PEG buffer (30% Polyethylene Glycol 8000, 30 mM $MgCl_2$), followed by a 10 min centrifugation at 15 000 g. The pellet was rinsed with 70% ethanol, dried and resuspended in 10 µl of sterile MQ water for sequencing. Sequencing was in a 20 µl volume containing 8 µl of ABI dye terminator chemistry (Perkin Elmer, Melbourne), 0.05 µM of primer and 50 ng of PCR product. The sequencing reaction was in a FTS-1 Thermal sequencer (Corbett Research, Mortlake) with a program of 96°C for 10 s, 50°C for 15 s and 60°C for 4 min, for 25 cycles. Sequences were run on an ABI 373A DNA sequencer. Sequences were aligned using Sequence Navigator (Applied Biosystems Inc, Ver1 1994).

Results

Two gene fragments were sequenced in both directions for the construction of a molecular phylogeny of *Flindersia* (Scott *et al.*, in press). The two fragments were the ITS-1 spacer (including 68 bp of 18S rRNA gene and 20 bp of 5.8S rRNA) which is nuclear, and the intergenic spacer between *trn*L-*trn*F of the chloroplast. The ITS-1 being nuclear is biparentally inherited, while the chloroplast *trn*L-*trn*F would be maternally inherited, as chloroplasts are maternally inherited in most plants. Both DNA fragments were able to differentiate every species within the genus, with the exception of *F. pimenteliana* and *F. oppositifolia* (Scott *et al.* in press). The ITS-1 fragment in *F. pimenteliana* and *F. oppositifolia* was 311 base pairs long. Sequence divergence for ITS-1 ranged from 1.2-13.4% between species pairs, with the exception of *F. pimenteliana* and *F. oppositifolia* that were identical. Similar studies using ITS-1 have reported sequence divergences of 5-48.9% between species in the genus *Gentiana* (Yuan *et al.* 1996), and 0.7-21% between species pairs in *Fraxinus* (Jeandroz *et al.* 1997). Given that the resolution of the ITS sequence data has in this case defined all other species within the genus, in addition to describing intraspecific variation in five of the 17 species of *Flindersia*, it would support the assertion that *F. pimenteliana* and *F. oppositifolia* do not constitute discrete taxa.

The second fragment that was sequenced, was from the chloroplast, and was 372 base pairs long. The *trn* fragment, like the ITS fragment was able to distinguish all other species within *Flindersia*, with the exception of differentiating *F. pimenteliana* from *F. oppositifolia*. The chloroplast fragment was less variable than the nuclear fragment with 0.25–4.2% sequence divergence between *Flindersia* species pairs. Sequence divergence between species pairs of *Alnus* have ranged from 0.87–1.52% with divergence values in *Fraxinus* ranging from 0.65–1.14% (Gielly and Taberlet 1994).

The genetic evidence provided from two independent gene fragments, would suggest that *F. pimenteliana* and *F. oppositifolia* do not show a level of genetic diversity which would be indicative of species recognition within *Flindersia*.

Systematics

Whilst different in habit and adult leaf form, both Hartley (1969) and Whiffin (1982) pointed out the similarities between *F. pimenteliana* and *F. oppositifolia* in the characters of the fruit, seed and seedlings and regarded both as being closely similar and Whiffin (*op. cit.*) suggested that *F. oppositifolia* (as *unifoliata*) was a 'highly derived montain form of *F. pimenteliana*' and his figure four showing the phylogeny of the genus places the two species in the one clade. Based on the observations of these authors and the molecular evidence presented above, we believe that the status of *F. oppositifolia* should be reassessed and make the following recombination.

Flindersia pimenteliana F.Muell. **forma oppositifolia** (F.Muell.) K. D. Scott, W. K. Harris & J.Playford, **comb. & stat. nov.**; *Hypsophila oppositifolia* F.Muell., Vict. Nat. 9: 11 (1892). **Type:** [Queensland, Cook District]: Mt Bartle Frere, 1892, *Johnson* (lecto: MEL, *fide* Hartley & Jessup (1982)) *n.v. Flindersia oppositifolia* (F.Muell.) Hartley & Jessup, Brunonia 5: 109 (1982).

Flindersia unifoliata Hartley, J.Arnold Arb. 50: 498 (1969); **Type:** [Queensland, Cook District]: Mt Bellenden Ker, *Sayer 136,* (holo: MEL *n.v.*).

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Table 1: Genbank and herbarium accession numbers for ITS-1 and *trn*L-*trn*F

Taxon	Herbarium Accessions or sample origin	ITS-1 Genbank Accessions	*trn*L-*trn*F Genbank Accessions
Flindersia oppositifolia	BRI AQ 522053 BRIAQ 484238 BRIAQ 459477	AF025500*	AF026021*
Flindersia pimenteliana	BRIAQ 522064 BRIAQ 522058 Australian National Botanic Gardens	AF025501*	AF026022*

* Note that there is only a single genbank submission for the herbarium accessions, as the sequences for each species were identical.